IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant(s): He, et al. Application No.: 10582820 Filed: 10/8/2008 Title: Single Molecule Detection Using Molecular Motors Attorney Docket No.: 60224US	Confirmation No. **7416** Art Unit: 1634 Examiner: SISSON, BRADLEY L

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

THIRD PROPOSED RESPONSE UNDER 37 CFR 1.111 FOR DISCUSSION PURPOSES

Dear Commissioner:

Applicants extend their thanks to Examiner Sisson for courtesies extended during our second telephone interview of June 21, 2011 and for agreeing to schedule a second telephone interview in this matter on June 28, 2011 at 4 pm ET. In response to the Office Action of April 6, 2011, the following amendment is presented for discussion purposes.

Amendments to the Claims begin on page 2 of this paper.

Remarks/Arguments begin on page 7 of this paper.

Amendments to the Claims:

This listing of claims will replace all prior versions, and listings, of claims in the application:

Listing of Claims:

Claim 1 (currently amended): A method for detecting ~~a~~ at least one target nucleic acid comprising:

(a) providing at least one set of first and second target-specific nucleic acids, wherein the at least one set of first and second target-specific nucleic acids each comprise nucleotide sequences complementary to a specific one of the at least one target nucleic acid; wherein each of the at least one set of first and second target-specific nucleic acids are specific only for a selected one of the at least one target nucleic acid; wherein each first target-specific nucleic acid is bound to a selected first affinity tag and each second target-specific nucleic acid is bound to a selected second affinity tag, wherein the selected first affinity tag is capable of binding specifically to a molecular motor, wherein the molecular motor is a biological or synthetic molecule capable of induced translational or rotational movements that are capable of being detected, wherein the selected second affinity tag is capable of binding specifically to a metal nanorod detection probe;

(b) contacting the at least one set of first and second target-specific nucleic acids to a sample under conditions whereby the at least one set of first and second target-specific nucleic acids will only hybridize to the at least one target nucleic acid if the at least one target nucleic acid is present in the sample, wherein the target nucleic acid and the first and second target-specific nucleic acids' nucleotide-base-pairing-specific-ligation reaction requires formation of ~~upon hybridization to the target nucleic acid the first and second target-specific nucleic acids are~~ juxtaposed ~~at~~ 5' phosphate and 3' hydroxyl termini of two adjacent target-specific nucleic acids which are hybridized to the complementary target nucleic acid to form a nucleic acid strand that contains a first affinity tag and a second affinity tag at the 5' and 3' ends respectively;

(c) upon hybridization to the at least one target nucleic acid, ligating the first and second target-specific nucleic acids together;

(d) binding one of a series of molecular ~~motor~~ motors to the selected first affinity tag ~~and the detection probe to the second affinity tag~~;

(e) binding the series of molecular motors on a solid support either before or after assembly with nucleotide containing affinity tags on the 5' and 3' ends;

(f) binding the metal nanorod detection probe to the selected second affinity tag of the molecular motor-target nucleotide complex either before or after the series of molecular motors is bound to the solid support;

([[f]] g) inducing translational or rotational movement of at least one of the molecular motors; and

([[g]] h) detecting translational or rotational movement of the at least one molecular motor coupled to the solid support ~~through the detection probe~~ ~~regularly~~ as indicated by ~~changing~~ ~~color~~ monitoring changes in light intensity of at least one wavelength originating from the metal nanorod detection probe, where the at least one wavelength indicates the presence of a unique corresponding target nucleic acid in the sample, or by observing the metal nanorod detection probe translationally moving wherein differing selected colors of each of the translationally moving metal nanorod detection probes indicate the presence of a unique corresponding target nucleic acid ~~wherein the motor movement of the molecular motor serves to detect the target nucleic acid~~ in the sample~~, and where observation of ATP-dependent rotation of different colored nanorods indicates the presence of a corresponding target nucleic acid each having its unique probe attachment or different motors causing different specific motor-induced motion so as to allow determination of an assortment of different target nucleic acid(s) is/are present in any given sample~~.

Claim 2 (currently amended): The method of claim 1 wherein the method further comprises generating a plurality of target and probe nucleotide base-pairing specific ligation products following step (c) using ligation chain reaction.

Claim 3 (currently amended): The method of claim 1 wherein the molecular motor comprises ~~consists essentially of~~ ~~an~~ F1-ATPase.

Claims 4-7 (canceled).

Claim 8 (new): The method of claim 1 wherein monitoring comprises monitoring oscillation of intensity of light of only one wavelength.

Claim 9 (new): The method of claim 1 wherein detecting translational or rotational movement of the at least one molecular motor coupled to the solid support comprises attaching the molecular motor onto a nano-electrode and measuring the micro current change or impendence change produced by rotation.

Claim 10 (new): A method for detecting at least one target nucleic acid comprising:

(a) providing at least one set of first and second target-specific nucleic acids, wherein the at least one set of first and second target-specific nucleic acids each comprise nucleotide sequences complementary to a specific one of the at least one target nucleic acid; wherein each of the at least one set of first and second target-specific nucleic acids are specific only for a selected target nucleic acid; wherein each first target specific nucleic acid is bound to a selected first affinity tag and each second target-specific nucleic acid is bound to a selected second affinity tag, wherein the selected first affinity tag is capable of binding specifically to a molecular motor, wherein the molecular motor is a biological or synthetic molecule capable of induced translational or rotational movement that are capable of being detected, wherein the selected second affinity tag is capable of binding specifically to a metal nanorod detection probe;

(b) contacting the at least one set of first and second target-specific nucleic acids to a sample under conditions whereby the at least one set of first and second target-specific nucleic acids will only hybridize to the at least one target nucleic acid if the at least one target nucleic acid is present in the sample, wherein the target nucleic acid and the first and second target-specific nucleic acids' nucleotide-base-pairing-specific-ligation reaction requires formation of juxtaposed 5' phosphate and 3' hydroxyl termini of two adjacent target-specific nucleic acids which are hybridized to the

complementary target nucleic acid to form a nucleic acid strand that contains a first affinity tag and a second affinity tag at the 5' and 3' ends respectively;

(c) upon hybridization to the at least one target nucleic acid, ligating the first and second target-specific nucleic acids together;

(d) binding one of a series of molecular motors to the selected first affinity tag;

(e) binding the series of molecular motors on a solid support either before or after assembly with nucleotide containing affinity tags on the 5' and 3' ends;

(f) binding the metal nanorod detection probe to the selected second affinity tag of the molecular motor-target nucleotide complex either before or after the series of molecular motors is bound to the solid support;

(g) inducing translational or rotational movement of at least one of the molecular motors; and

(h) microscopically detecting translational or rotational movement of the at least one molecular motor coupled to the solid support as indicated by monitoring changes in light intensity of at least one wavelength originating from the metal nanorod detection probe, where the at least one wavelength indicates the presence of a unique corresponding target nucleic acid in the sample, or by observing the metal nanorod detection probe translationally moving wherein differing selected colors of each of the translationally moving metal nanorod detection probes indicate the presence of a unique corresponding target nucleic acid in the sample.

Claim 11 (new): The method of claim 10 wherein microscopically detecting comprises using a microscopy technique selected from the group consisting of dark field microscopy and atomic force microscopy.

Claim 12(new): The method of claim 10 further comprising attaching a fluorescent label on a non-rotating part of the molecular motor before microscopically detecting translation or rotational movement, where the metal nanorod detection probe is a quencher metal nanorod detection probe, and wherein microscopically detecting translational or rotational movement comprises observing rotation through periodic quenching of a fluorescence signal by the quencher metal nanorod detection probe.

Claim 13 (new): The method of claim 1 further comprising attaching a fluorescent label on a non-rotating part of the molecular motor before detecting translation or rotational movement, where the metal nanorod detection probe is a quencher metal nanorod detection probe, and wherein detecting translational or rotational movement comprises observing rotation through periodic quenching of a fluorescence signal by the quencher metal nanorod detection probe.

Claim 14 (new): The method of claim 1 wherein detecting translational or rotational movement of the at least one molecular motor coupled to the solid support comprises using a detection technique selected from the group consisting of dark field microscopy, single molecule fluorescence resonance energy transfer, fluorescence lifetime anisotropy, atomic force microscopy, single molecule anisotropy measurement, and using a surface plasmon resonance biosensor to measure the surface plasmon resonance change during metallic nanorod rotation.

REMARKS

Claim Objections

The office has objected to Claims 5-7 under 37 CFR 1.75(c), as being of improper dependent form for failing to further limit the subject matter of a previous claim. In response claims 4-7 have been canceled. New claims 8-14 have been added. Applicant respectfully submits that the new and amended claims comply with 37 CFR 1.75(c) and are now in condition for allowance. Consideration of the amended claims is requested.

Claim Rejections - 35 USC § 112

Claim(s) 1-7 are pending in the application. Claims 1-7 stand rejected under 35 U.S.C. 112, first paragraph, as failing to comply with the enablement requirement. Claims 4-8 have been canceled. Applicant respectfully traverses the rejection of claims 1-3 in view of the arguments and amendments herein.

Applicants have made a diligent effort to place the claims in condition for allowance. However, should there remain unresolved issues that require adverse action, it is respectfully requested that the Examiner telephone George A. Leone, Applicants' Attorney at 253-682-0246 so that such issues may be resolved as expeditiously as possible.

For these reasons, and in view of the above amendments, this application is now considered to be in condition for allowance and such action is earnestly solicited.

Respectfully Submitted,

June 21, 2011
Date

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